

# PREDICTIVE SIGNIFICANCE OF SERUM INTERLEUKIN-10 FOR ACUTE GRAFT VERSUS HOST DISEASE PRIOR TO ALLOGENEIC BONE MARROW TRANSPLANTATION

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**Abstract-** Interleukin-10 (IL-10) is a potent immunosuppressor which inhibits alloantigenic T cell response. IL-10 is also an anti-inflammatory endogenous cytokine. To investigate the predictive significance of endogenous IL-10 for acute graft versus host disease (GVHD) following allogeneic bone marrow transplantation (BMT), we performed a prospective study on spontaneous IL-10 production in the serum of 96 patients admitted for allogeneic BMT. High spontaneous IL-10 production at the time of admission and prior to any preparative treatment correlates with a subsequent low incidence of GVHD as compared to patients with low IL-10 production. Our data demonstrate the predictive significance of increased IL-10 production in BMT patients and suggest a role for it in maintaining immunobalance in the setting of allogeneic BMT.

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**Key words:** Bone marrow transplantation, interleukin-10, graft versus host disease, bone marrow transplantation

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## INTRODUCTION

It has been shown that in the course of pretransplant conditioning, interleukin-10 (IL-10) is involved in modulation of graft versus host disease (GVHD) in patients receiving bone marrow transplantation (BMT) (1). In the world of cytokines, activation of proinflammatory mediators is followed by increased production of endogenous inhibitory molecules including antagonistic cytokines. Among endogenous antagonists, IL-10 has attracted much

attention. IL-10 is produced by T cells, including T-helper (Th)0, Th1 and Th2 subgroups, monocytes and macrophages. IL-10 possesses a variety of biological characteristics. Production of proinflammatory monokines is suppressed by IL-10 (2). It also modifies the expression of important molecules like CD80 on antigen presenting cells (APC) (3). IL-10 can also inhibit alloantigen-induced T cell activation (4).

It can be concluded that IL-10 might be a suppressive mediator involved in preventing acute GVHD and in inducing T cell tolerance in BMT. However, some studies using recombinant IL-10 as an antagonist *in vivo* revealed no beneficial effects.

We performed this study to evaluate the predictive significance of endogenous IL-10 for acute GVHD.

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## MATERIALS AND METHODS

This study was performed prospectively on the patients who received allogeneic BMT from November 2001 up to August 2003. The setting was Hematology, Oncology and BMT Research Center, Shariati General Hospital, Tehran University of Medical Sciences, Tehran, Iran. Patients with allogeneic BMT were included and those with autologous BMT were excluded. A consecutive series of 96 patients were evaluated. All of the patients were transplanted from HLA-identical sibling donors. Bone marrow was used as a stem cell in all patients. Regarding ethical issues, the study was approved by ethical committee of the Hematology, Oncology and BMT Research Center. Collection of blood was explained to all patients and relatives, and informed consent was obtained at the admission. Personal information considered confidential.

All of the patients were followed for 100 days posttransplantation, beginning from the first day of bone marrow transplantation, and evaluated for the occurrence of grades 3 or 4 acute GVHD as end point. Clinical staging and grading of acute GVHD were based on criteria shown in table 1. Patients were followed by hematologist and oncologist attending physicians who were unaware of the patients' serum IL-10. They filled in the International Bone Marrow Transplant Registry (IBMTR) core form (Series 095 Reporting Forms)

for each patient which was used as the source of the required information in this study.

Pretransplant conditioning (busulfan/cyclophosphamide) and prophylaxis (cyclosporine and a short course of methotrexate) was performed for each patient and treatment (prednisolone as the first-line medication) for acute GVHD and supportive care was done as indicated.

Blood was obtained at the time of admission, prior to the application of any cytotoxic drugs. The assay was based on the "quantitative enzyme-immunoassay" principle, using two monoclonal antibodies from mouse, directed against two different epitopes of IL-10. During the first incubation step, IL-10 in standards/samples is simultaneously bound by the biotin-labeled antibody and the peroxidase conjugated detection antibody, forming a complex which binds via the biotin-labeled antibody to the streptavidin-coated surface of the micro titer plate (one-step-system). Subsequently, the peroxidase bound in the complex is developed by tetramethylbenzidine as a substrate and is detected photometrically. The developed color is proportional to the concentration of IL-10. Standards of defined concentrations are run in each assay, allowing the construction of a calibration curve by plotting absorbance versus concentration. The IL-10 concentration of unknown sample is then calculated from this calibration curve.

Plasma IL-10 levels were determined for all patients at the time of admission by a person who did not know the patients and was not involved in the clinical management of the patients.

**Table 1.** Clinical staging and grading of acute graft-versus-host disease

Clinical stage	Skin	Liver-Bilirubin (mg/dl)	Gut
1	Rash <25% body surface	2-3	Diarrhea 500-100 ml/d
2	Rash 25-50% body surface	3-6	Diarrhea 100-1500 ml/d
3	Generalized erythroderma	6-15	Diarrhea >1500 ml/d
4	Desquamation and bullae	> 15	Ileus
Overall clinical grade	Skin stage	Liver stage	Gut stage
I	1-2	0	0
II	1-3	1	1
III	1-3	2-3	2-3
IV	2-4	2-4	2-4

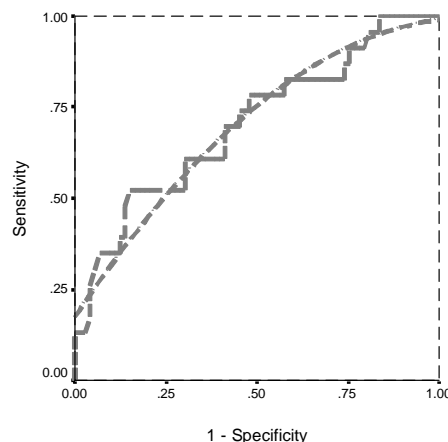
After BMT was performed and all the patients were followed for 100 days, all of the patients were classified to group 1 (including patients with grades 1 or 2 of acute GVHD) and group 2 (including patients with grades 3 or 4 of acute GVHD) as determined by clinical diagnostic criteria for staging and grading of acute GVHD. In addition to IL-10, several other factors including recipient's gender and underlying diagnosis were also analyzed for acute GVHD.

Statistical analysis was done by SPSS-10. Receiver operating characteristic (ROC) curve, its standard error (SE), and 95% confidence interval (CI 95%) were calculated. Normality assumption of serum IL-10 was evaluated by Kolmogorov-Smirnov test. Comparison of serum IL-10 between group 1 and 2 was done by Mann-Whitney U test. Chi square was used for qualitative variables (like recipient's gender and underlying diagnosis in relation to GVHD). Exact *P* value was calculated if indicated. *P* value < 0.05 was considered as significant. Since all of the donors and recipients were HLA full-matched, HLA compatibility was not selected as an independent variable.

## RESULTS

Among 96 patients whom were studied, 57 (59.4%) were male and 39 (40.6%) were female. Mean age of them was 25±8 years. Patients' underlying diseases included acute myeloid leukemia in 19 (19.8%), chronic myeloid leukemia in 20 (20.8%), acute lymphoblastic leukemia in 16 (16.7%), thalassemia in 28 (29.2%), aplastic anemia in 8 (8.3%), non-Hodgkin lymphoma in 1 (1%) and myelodysplastic syndrome in 4 (4.2%) patients.

In all of the study subjects, mean (± SE) of serum IL-10 was 112.47 (± 30.73) pg/ml. The minimum and maximum values were 0.48 pg/ml and 1634 pg/ml, respectively. In 23 (24%) of subjects, acute GVHD of grade 3 or 4 occurred during 100 days of follow up (Group 2). Based on Kolmogorov-Smirnov test, distribution of serum IL-10 was not normal (*P* < 0.001). Mean (± SD) of serum IL-10 in group 1 patients (including patients with acute GVHD grade of 1 or 2) was 140.91 (±339.78) pg/ml



**Fig.1.** Receiver operating characteristic (ROC) curve for prediction of the occurrence of acute graft versus host disease in the patients based on serum IL-10 level at the time of admission.

and in group 2 (including patients with acute GVHD grade of 3 or 4) was 22.18 (±49.06) pg/ml (Mann-Whitney U= 498.5, Z= -2.92, *P*= 0.003). Area under the ROC curve was 0.703 ± 0.065 (SE) with CI 95%: 0.576-0.830 and *P* value = 0.003 (Fig.1).

Table 2 represents sensitivity and specificity of different amount of serum IL-10 level at the time of admission for occurrence of acute GVHD in group 2 (including patients with grades 3 or 4 of acute GVHD) after 100 days post transplantation follow up in 96 patients.

There was no statistically significant association between gender of the patients and occurrence of acute GVHD ( $\chi^2 = 0.02$ , df = 1, *P* = 0.87). Also there was no significant association between underlying disease and occurrence of acute GVHD ( $\chi^2 = 6.9$ , df= 6, exact *P* = 0.33).

**Table 2.** Sensitivity and specificity of various levels of serum IL-10 at the time of admission for occurrence of acute GVHD in group 2 (including patients with grades 3 or 4 of acute GVHD) after 100 days post transplantation follow up in 96 patients.

Serum IL-10 level		
(pg/ml)	Sensitivity	Specificity
3	34%	90%
57.5	91%	22%
241	100%	13%

Abbreviation: graft versus host disease

## DISCUSSION

Graft-versus-host disease (GVHD) is a direct result of one of the principal functions of the immune system: the distinction of self from non-self (5). The basic requirements for the development of this disorder were recognized as early as the 1960s (6). These include the following characteristics:

- The graft must contain immunologically competent cells.

- The host must possess transplantation antigens that are lacking in the graft, thereby appearing foreign to the graft; host cells subsequently stimulate donor cells via these specific antigenic determinants.

- The host must be incapable of mounting a reaction against the graft for a period of time sufficient to allow graft cells to attack the host (5).

Since GVHD is primarily a T cell mediated disease, this discussion of the pathogenesis of the disorder consists of an overview of the more important properties and interactions of transplanted T cells which may lead to the disease (5). Once a T cell is activated and begins to proliferate, it releases a variety of pro-inflammatory cytokines (7). Cytokines may be very important in various steps of the graft-versus-host reaction. These include the initial stimulation of T cells, the maintenance of stimulation, the afferent phase and the efferent phase, a period when toxicity is manifested (5).

Our study revealed an association between increased IL-10 production at the time of admission with subsequent lower grades of acute GVHD (grade  $\leq 2$ ) and relatively uneventful courses which suggest a protective role of IL-10. It may be due to down-modulation of proinflammatory cytokine production by IL-10 (8). High levels of IL-10 produced prior to BMT may down-regulate tissue expression of intercellular adhesion molecules (ICAM) and ICAM-1-mediated transendothelial migration of inflammatory cells which occurs after irradiation or bacterial endotoxin activation (9, 10). In addition, down modulation of other costimulatory molecules including CD80 on antigen presenting cells such as dendritic cells by IL-10 may result in tolerance (11). Finally, presence of high levels of IL-10 at the time of donor cell infusion may induce a host-specific antigen state in donor T lymphocytes as reported *in vitro* in mixed lymphocyte reactions carried out in the presence of IL-10 (3).

In summary, current data suggest a major role of IL-10 in maintaining immunobalance in the setting of BMT and are in accordance with previous studies reporting an association of tolerance with increased IL-10 production in patients with severe combined immunodeficiency receiving HLA mismatched grafts (12). Besides the possible systemic effects of increased IL-10 production by recipient cells, the generation of tolerizing regulatory donor T cells can be responsible for the phenomenon (8, 13).

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